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Snapback high resolution melting assays for rapid and inexpensive detection of varroa treatment resistance markers for pyrethroids and amitraz

John Mackay, Noa Smith, Rebecca Hewett
dnature diagnostics & research Ltd, Gisborne, New Zealand

Genetic resistance of Varroa destructor to pyrethroid treatments has been well documented over the past decade. Originally described as the mutation L925V in the voltage-gated sodium channel of varroa, this single amino acid has been shown to mutate in 3 different forms (L925V, L925M and L925I). These variants vary geographically with L925V prevalent in Europe and L925M widespread in the USA. However, to detect and differentiate all three variants has typically required the use of variant-specific and expensive qPCR hydrolysis (TaqMan) probes, while simpler PCR-based methods have been used to detect only a single mutation per reaction.

These variant-specific probes have also been employed to detect the more recently discovered mutations in varroa that confer resistance to amitraz-based treatments. These mutations occur in various locations on the newly identified target gene for amitraz, the beta2-octopamine receptor.

High resolution melting analysis (HRMA) and the newer snapback form of this method utilise two inexpensive primers in a PCR reaction which can discriminate DNA sequences down to SNP level, without the use of separate dye-labelled probes. Indeed, snapback HRMA uses a simple unlabelled additional sequence on the 5' end of one of the primers, that acts as a probe element by folding over or 'snapping back' to bind to the SNP region of interest. Analysis is performed by monitoring the different melting curves between wild-type and mutated sequences. We demonstrate novel test designs that discriminate multiple mutations (in the instance of the multiple mutation L925 hotspot) in a single reaction or individual mutation reactions for the multiple amitraz resistance regions.

Combined with a rapid, single tube DNA-release buffer for individual varroa; these assays provide a rapid and inexpensive means to monitor the occurrence of chemical resistance in varroa as well as rapidly implement inexpensive new designs as novel mutations are discovered. Despite widespread use of registered pyrethroid and amitraz-based products in New Zealand since the introduction of Varroa destructor in 2000, no genetic resistance in the voltage-gated sodium channel (pyrethroids) or beta2-octopamine receptor (amitraz) has been confirmed in the varroa samples analysed to date.